Identification of New Major Histocompatibility Complex Class II Restriction Fragment Length Polymorphisms in a Closed Experimental Line of Beltsville Small White Turkeys

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ABSTRACT Beltsville Small White (BSW) turkeys have been utilized as an experimental model in the study of bacterial, parasitic, and fungal diseases. Given the critical role of MHC antigens in the initial steps of the immune response to specific pathogens, the MHC Class II of BSW turkeys was characterized. Southern blot analysis of *Pvu*II-digested turkey DNA that was hybridized with a chicken Class II β gene genomic clone revealed two restriction fragment length polymorphism profiles not previously identified in experimental or commercial breeder lines of turkeys. These fingerprint profiles differed in a single 6.0-kb band that was present in approximately 38% of the birds examined. The DNA fragments of 5.0, 4.1, 3.3, and 3.1 were present in both profiles. Furthermore, no mixed lymphocyte reaction was observed between individuals within the BSW turkey line. The present results indicate that BSW turkeys represent a unique source of genetic diversity for MHC Class II haplotypes.

(*Key words*: turkey, major histocompatability complex, haplotype, genetic diversity, restriction fragment length polymorphism)

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INTRODUCTION

The MHC is a chromosomal region containing a family of genes that encode a group of glycoproteins involved in regulating a number of cellular processes of importance to the immune response. The chicken MHC, the B complex, was originally designated as a blood group locus (Briles et al., 1950). Three classes of genes have been identified for the B complex, Classes I (B-F), II (B-L), and IV (B-G) (Pink et al., 1977). The functions of chicken MHC Class I and II gene products are similar to those of human and mouse, including involvement in phenomena such as graft-versus-host reaction (GVHR), mixed lymphocyte reaction (MLR), skin graft rejection, cell-to-cell recognition, and disease resistance. The Class IV genes are unique to avian species and encode antigens expressed on erythrocytes (Pink et al., 1977) and intestinal epithelium (Miller et al., 1990).

Genotyping by restriction fragment length polymorphism (RFLP) analysis has proven to be a powerful method for the identification of MHC polymorphisms in avian species. The RFLP analysis can be used to type each MHC class specifically. At least seven haploytpes of MHC Class II genes have been identified in turkeys (Emara et al., 1993; Zhu et al., 1995). These haplotypes were identified in four experimental turkey lines utilized in genetics studies as well as in primary breeding lines of commercial turkeys.

Functional tests have been utilized to distinguish differences in histocompatibility among chickens and turkeys. These reactions include skin graft rejection, GVHR, and MLR (Schierman and Nordskog, 1961; Schou, 1980; Simonsen, 1985; Emara et al., 1993). It has been hypothesized that chicken MHC Class I molecules have a predominant role in skin graft rejection (Hala et al., 1976), whereas MHC Class II molecules regulate the MLR (Crone et al., 1981).

Beltsville Small White (BSW) turkeys have been used by several investigators in the study of infectious diseases in turkeys (Edgar and Flanagan, 1979; Barnes and Hofstad, 1983; Augustine, 1988; Rimler and Kunkle, 1997). It has been well established that polymorphisms of MHC genes are important in disease resistance of animals. Because the MHC Class II of BSW turkeys has not been previously characterized, the objectives of the present study were to use RFLP analysis to characterize MHC Class II genes and the MLR to determine whether there are functional differences in the MHC Class II of BSW turkeys.

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Abbreviation Key: BSW = Beltsville small white (turkey); GVHR = graft-versus-host reaction; MLR = mixed lymphocyte reaction; RFLP = restriction fragment length polymorphism; SI = stimulation index.

MATERIALS AND METHODS

The BSW and a commercial line of turkeys were used in the present experiments. The BSW turkey was developed by the United States Department of Agriculture (Marsden, 1967). Several breeds of turkeys available at the time of development were used in producing the BSW. Introduced at various times in the breeding program were Narragansetts, "Baby Beef" Bronze, White Hollands, wild turkeys, Charlevoix, Black, White Austrian, and Broad Breasted Bronze. The BSW was recognized as a new standard variety in 1951 and first appeared in the 1953 American Standard of Perfection. An experimental line of BSW turkeys has been maintained as a closed flock at the National Animal Disease Center since 1962.

RFLP Analysis

The RFLP analysis method has been reported previously (Emara et al., 1992; Zhu et al., 1995). Briefly, genomic DNA was isolated from turkey blood cells, digested with *Pvu*II restriction enzyme, and separated on a 0.8% agarose gel for 30 h at 1.5 V/cm. Denatured fragments were blotted onto nylon membranes. Prehybridization and hybridization were carried out following the recommendations of the manufacturer.[2] A 2.3-kb fragment from a genomic clone of a chicken MHC Class II β gene was used as the probe (Xu et al., 1989).

Isolation of Turkey Peripheral Blood Lymphocytes

Peripheral blood was collected into vaccutainer tubes containing EDTA as the anticoagulant.[3] Lymphocytes were isolated using Ficoll-Hypaque gradient centrifugation.[4] The isolated mononuclear cells were washed two times in RPMI 1640[5] before use in a two-way mixed lymphocyte reaction.

Mixed Lymphocyte Reaction

A two-way mixed lymphocyte reaction was performed using a procedure similar to that previously described (Emara et al., 1993). Briefly, 100-μL aliquots of cells (2.5×10^6/mL) from each of two birds were added to wells of a 96-well round bottom plate.[6] The lymphocyte cultures were incubated at 37 C for 120 h in RPMI 1640 supplemented with 2 m*M* L-glutamine, $NaHCO_3$, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2.5% conditioned serum replacement supplement-2.[4] Twenty-four hours prior to harvest, cells were pulsed with 1 μCi of [^{3}H]thymidine. At 120 h of incubation, cells were harvested by aspiration onto glass fiber filter paper using using a Tomtec Harvester 96-cell harvester.[7] Filters were counted in a scintillation counter. A stimulation index (SI) was determined for each two-way mixed lymphocyte reaction by dividing the counts per minute from cells of A and B combined by the average counts per minute of A (alone) plus counts per minute of B (alone), where A and B represent two individual birds. Student's *t*-test was used to determine whether values were statistically significant.

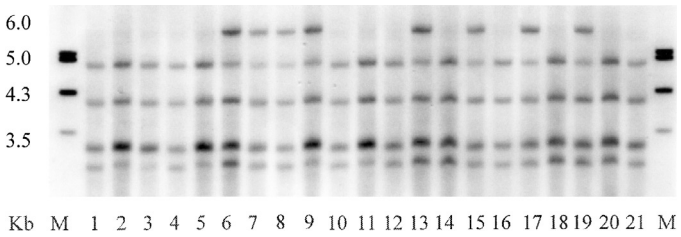

FIGURE 1. Southern blot analysis of *Pvu*II-digested turkey DNA hybridized with a nonradioactive probe made from a chicken MHC Class II genomic clone. Lanes 1 to 21 represent restriction fragment length polymorphism patterns of 21 individual Beltsville Small White turkeys. Lanes marked M contain the molecular size markers.

RESULTS

In the closed flock of BSW turkeys used, two RFLP patterns were detected that were unique when compared to the fingerprint profiles previously reported (Figure 1). These fingerprint profiles differed in that a 6.0-kb band was present in approximately 38% of the birds. Fragments of 5.0, 4.1, 3.3, and 3.1 kb were observed in *Pvu*II-digested DNA samples from all individuals examined.

In order to determine if there are functional differences among MHC Class II antigens in BSW turkeys, a two-way MLR was conducted. Responding and stimulating cells were prepared from 15 BSW turkeys (two experiments), and paired comparisons were made in a two-way MLR. As shown, SI values among individuals of the BSW line were less than 3.0 (Table 1) and were not significantly different from self MLR. Similar results for MLR were obtained in both experiments. In contrast, when cells from six Broad Breasted White turkeys from a commercial line were used in a MLR with cells from BSW turkeys, stimulation indices were generally between 4.0 and 7.1.

DISCUSSION

Previously, at least 22 MHC Class II RFLP patterns have been observed in *Pvu*II-digested DNA samples from 4 experimental lines, 11 primary breeding lines, and wild turkeys (Emara et al., 1993; Zhu et al., 1995, 1996). Of the 22 fingerprint profiles, there are likely seven MHC Class II haplotypes represented. In the present study, two unique Class II RFLP profiles not previously observed were detected in DNA samples from individuals in a closed flock of BSW turkeys. The two patterns were identical except for the presence of a 6.0-kb fragment observed in 38% of the birds. Based on a two-way MLR, there are no apparent

[2]Boehringer Mannheim, Indianapolis, IN 46250.
[3]Becton Dickson, Franklin Lakes, NJ 07417.
[4]Sigma Chemical Co., St. Louis, MO 63178.
[5]Gibco BRL, Life Technologies, Inc., Gaithersburg, MD 20877.
[6]Dynatech Laboratories, Chantilly, VA 22021.
[7]Wallac, Inc., Gaithersburg, MD 20877.

TABLE 1. Stimulation index values from two-way mixed lymphocyte reactions among turkeys of the Beltsville Small White line[1,2]

Bird number	218	222	2376	2450	2593	3838	3846
198	1.06	1.59	2.64	2.24	1.03	1.45	1.68
218		1.34	1.32	0.90	0.80	0.96	1.01
222			1.06	1.45	0.91	2.45	1.23
2,376				0.86	2.26	1.48	1.30
2,450					1.84	2.25	1.52
2,593						1.31	0.78
3,838							1.07

[1]Stimulation index = counts per minute of cells from A + B divided by the average of the counts per minute of cells from A and B alone, where A and B are individual birds. Results shown are from one representative experiment.

[2]Cells (2.5×10^5) from each bird were placed in 96-well round bottom plates and were cultured for 120 h.

functional differences between the two RFLP patterns observed in BSW turkeys.

The BSW turkeys have been utilized as an experimental model in the study of a number of infectious diseases. Given the critical role of MHC antigens in the immune response to specific pathogens, the present results, showing unique polymorphisms in MHC Class II indicate that BSW turkeys may exhibit an altered specific immune response to certain diseases as compared to commercial turkeys. However, based on the results in the present study, BSW turkeys could be useful as a model in experimental situations that require the use of Class II syngeneic cells.

Diversity of MHC Class II haplotypes in commercial turkeys is low in comparison to wild turkeys (Zhu et al., 1996). Eight of 11 commercial lines examined had no more than four haplotypes, and loci in some lines were close to fixation. Zhu et al. (1996) suggested that in order to increase the diversity of MHC haplotypes in commercial turkeys, introgression may be more efficient that within-line selection. These authors proposed that their experimental lines of turkeys, with greater observed diversity in MHC Class II genes, could serve as a source of genetic variation. The present results indicate that BSW turkeys represent a unique source of variation in MHC Class II genes that could be exploited in the future.

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